

EMERSION OF THE AMPHIBIOUS CHILEAN CLINGFISH, *SICYASES SANGUINEUS*

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Amphibious fishes “. . . spend periods of time out of water, on or above the ground surface, as normal parts of their life histories” (Gordon, Böetius, Evans, McCarthy, and Oglesby, 1969, page 141). Many inhabit marginal zones like the marine intertidal zone, which fluctuates between aquatic and terrestrial conditions. Some gobies and blennies briefly expose themselves as they scurry from one tidal pool to another; others may remain out of water for many hours while the tide is out, keeping moist in damp seaweed or under rocks (Gordon, 1966). William H. Eger (in preparation) found several small species of clingfishes (Gobiesocidae) of the Gulf of California in moist areas under dry rocks or in clumps of snails far from the water's edge.

Sicyases sanguineus of coastal Chile and southern Perú is an exceptionally large clingfish which can live both below the cool and well-aerated surf and above water on exposed rocks (Buen, de, 1960). This Chilean clingfish attaches by means of a large disc formed of the fused and highly modified pelvic fins, bearing small and flattened dermal papillae (Briggs, 1955). The complementary forces of suction by the disc and adhesion by the papillae secure this clingfish to the rock, so that it resembles a large chiton or limpet blending with the dark substrate. Although young fish are relatively active as they occasionally scamper in and out of the water, adults are quiescent as they cluster on exposed rocks often well above water level (Vargas and Concha, 1957ab).

To survive above the surf, clingfish must (1) either tolerate large fluctuations of the environment or remain in the splash zone and (2) breathe air. If they leave the splash zone, they risk variable oxygen concentration, temperature, and salinity in isolated tidal pools, and desiccation and overheating on drying rocks (cf. Carter, 1931). If emerged fish were continuously wetted and cooled in the splash zone, however, they could survive with minimum adjustments, other than those necessary for breathing air. With this in mind, we set out: (1) to determine the optimal circumstances of the Chilean clingfish's life above water; (2) then, to find out why these circumstances generally prevail and if they can be largely compromised; which (3) led to experimental studies of the mechanism of the fish's aerial respiration. Our investigations supplemented more intensive physiological studies by Gordon, Fischer, and Tarifeño (in preparation) of the survival of the fish out of water.

MATERIALS AND METHODS

Field observations

We observed the behavior and distribution of fish at three localities along the coast of central Chile, north of Valparaíso in a subtropical region of light to moderate rainfall. At each locality fish were watched through binoculars as they clung to the vertical surfaces of three large rocks on progressively higher ground: rock 1 with base awash at low tide, to rock 3 with base awash only at high tide. These surfaces faced mostly southwest, so that they were shaded in the morning and sunlit in the afternoon. They either paralleled the surge or formed a lee, protecting the fish from the full force of the surf. Locality I was in a relatively urban area just north of the Estación de Biología Marina at Montemar, while localities II and III were in sparsely populated rural areas about 60 km north of I.

The observed surfaces of rocks 1 measured about four square meters at localities I and III, and about six square meters at locality II; rocks 2 and 3 together measured barely three square meters at all localities. The surface of rock 1 was subjectively divided into two habitats: a lower half generally wet at low tide and an upper half generally dry at low tide. A fringe of brown algae about one meter above tidal low underlaid the surfaces of rocks 1 and 2. With approaching high tide, larger swells broke over the rocks to form characteristic rivulets down the sheltered surfaces. Clingfish often congregated in these relatively wet areas.

The three localities were visited a total of 38 times between October 7 and November 20, 1967. A set of 24 observations was made during each visit. The diel distribution of visits was: 5 visits between 0720 and 0913 hrs, 11 visits between 1019 and 1308 hrs, 3 visits between 1340 and 1515 hrs, 8 visits between 1526 and 1723 hrs, 8 visits between 1700 and 1825 hrs, 2 visits between 1820 and 1905 hrs, and one visit between 2020 and 2100 hrs by spotlight. Localities II and III were visited only five times each.

Fifteen physical and nine biological variables were measured to relate emergent clingfish with their environment (Table I): *locality*, scored 1–3; *date*, in days from the first visit; *time of day* (hrs), 0700–2100; *wave height* (m), from trough to crest of swells about 25 m offshore; *water level*, scored 1–8, from minimum low tide in calm sea to maximum high in rough sea; *dryness, upper rocks*, scored 1–3, from the condition when all surfaces of the three rocks were wet to that when the upper halves of rocks 1 and 2 and all of 3 appeared dry; *relative humidity* (%) measured with a sling psychrometer; *air and water temperatures* (°C), water measured in the surf with bucket thermometer; *wind direction*, scored 1–4—NW, WNW, W, SW—and *velocity* (mph), recorded as the average of three measurements by hand-held anemometer; *overcast*, scored 1–10, from clear and sunny to complete overcast; *sun on rocks*, scored 1–3, from total shade, through hazy sun, to bright sun; *irradiance* (langlies/hr), measured in shade or sun, depending on the condition of light on the rocks, with an integrating photometer (Haley, 1967); *barometric pressure* (millibars), recorded off station from a barometer in the laboratory.

We observed fish from a distance of about 20–30 m; closer approaches disturbed them. Recorded abundances were the averages of three counts, which included all visible fish along or above the upper margin of the seaweed fringe on the rock surfaces. Numbers of *fish on rocks 1–3*, recorded separately, were also

summed to obtain *total fish*. Other observations were recorded for rock 1 only: the number of *fish* along the *seaweed fringe*, the per cent of *fish* on the *lower half* of the rock surface, the per cent of *fish* with *heads* pointed *down*, and the per cent of *juvenile fish* (young and halfgrown). The density of the *fish* in *crevices* and seaweed patches was scored 1-3 from scarce to abundant.

TABLE I
*Variables possibly affecting clingfish emerged in the rocky intertidal
of central Chile**

Variable	Range	Mean	Standard deviation	Communality
<i>Locality</i>	1-3	1.29	0.61	0.56
<i>Date</i>	1-51	28.1	13.6	0.23
<i>Time of day</i> (hrs)	815-2100	1396.	354.	0.53
<i>Wave height</i> (m)	0.6-2.75	1.33	0.67	0.60
<i>Water level</i> (score)	1-8	4.82	1.89	0.71
<i>Dryness, upper rocks</i> (score)	1-3	1.84	0.73	0.83
<i>Relative humidity</i> (%)	52-96	73.3	12.0	0.61
<i>Air temperature</i> (°C)	11.5-18.3	15.7	2.30	0.78
<i>Water temperature</i> (°C)	11.3-14.7	12.6	0.91	0.42
<i>Wind direction</i> (score)	1-4	2.68	1.27	0.50
<i>Wind velocity</i> (mph)	1.2-17.3	5.93	3.96	0.47
<i>Overcast</i> (score)	1-10	4.39	3.65	0.48
<i>Sun on rocks</i> (score)	1-3	1.87	0.85	0.64
<i>Irradiance</i> (langlies)	0-3.3	1.55	0.83	0.64
<i>Barometric pressure</i> (mb)	29.79-30.11	29.93	0.10	0.48
<i>Fish, total</i>	0-135.0	35.1	31.6	0.99
<i>Fish, rock 1</i>	0-131.3	26.0	30.0	0.99
<i>Fish, rock 2</i>	0-23.7	4.92	5.59	0.78
<i>Fish, rock 3</i>	0-18.0	4.19	4.56	0.66
<i>Fish, seaweed fringe</i>	0-12.1	2.26	2.56	0.50
<i>Fish on lower half of rock</i> (%)	0-100	80.4	28.1	0.48
<i>Fish with heads down</i> (%)	0-80	30.4	22.8	0.57
<i>Juvenile fish</i> (%)	41-100	93.9	14.6	0.67
<i>Fish in crevices, etc.</i> (score)	1-3	1.97	0.71	0.11

* Each variable, expressed by 38 observations, in defined in the text. The communality (0-1) measures interactions with the other variables.

Factors were computed to assemble groups of interacting variables into a few causal arrays, each defining a system of interactions either within the environment or between clingfish and environment (*cf.*, Sokal and Daly, 1961). First a 24 by 24 matrix of correlation coefficients, one coefficient for each pair of variables, was "factored" to extract latent roots and orthogonal vectors of 24 principal factors. Then a much smaller number of factors was obliquely rotated to "simple structure," so that fewer factors defined more natural groups of variables because these factors were no longer necessarily orthogonal to each other; *i.e.*, they could be inter-correlated (*e.g.*, Cattell, 1965; Harman, 1967). In this way, "factor analysis represents covariation by finding fewer dimensions of variation than the number of variables in a correlation matrix" (Thomas, 1968: 849). Because we had no *a priori* way of estimating the number of natural groups in the system of 24 variables, we compared three different representations composed of three, four, and

five rotated factors, respectively. The four-factor representation of the system appeared to be the most meaningful, because (1) two of the five factors were strongly intercorrelated (*cf.*, Thomas, 1968) and (2) three factors did not distinguish two important groups.

Each group was ordered into an array by decreasing magnitudes of the "loadings" of its several constituent variables on its factor (Table II). The loading of a variable was a measure of the variable's relative "importance" to, or correlation with, the factor, and was somewhat arbitrarily adjudged "significant" if it equaled or exceeded an absolute value of about 0.50 (Sokal and Daly, 1961).

Communalities estimated the proportion of the variation of each variable attributable to its covariation with the others (Cattell, 1965; Harman, 1967). Variables with high communalities interacted strongly with other variables. Variables with low communalities, however, explained relatively little of the system, in that a relatively large part of their variation was not attributable to their covariation with the others. This "error variance" was partitioned out of the analysis, so that it did not influence the factor loadings. The program BMDX72 for computation of the correlation matrix and communalities, extraction of 24 principal factors, and the subsequent rotation of a few factors to simple structure (Dixon, 1967) was modified for the IBM 360-75 computer at the University of California, Santa Barbara Computer Center.

Laboratory observations

Caught near localities I and II, experimental fish lived unfed in the laboratory in 40-liter plastic aquaria half-filled with aerated seawater of salinity about 34‰ at 12–17° C.

Responses of clingfish to aeration, stagnation, temperature, enforced emersion, and fresh water were noted of fish placed 1–3 at a time in covered 12-liter plastic aquaria. Seven of the fish were classified as adult (130–190 mm long), nine as halfgrown (100–120 mm), and two as young (60–90 mm). Submerged fish were observed in aerated seawater sun-warmed from 13 to 19° C or in seawater first aerated for 30 minutes, then allowed to stagnate in the shade. Emerged fish were observed on moist paper towels in an empty aquarium. In the two stagnating aquaria, body movements and opercular rates were recorded at 30-minute intervals during eight hours for three each of the halfgrown and adult fish as dissolved oxygen content, measured by galvanic-cell oxygen electrode, decreased from saturation. Six halfgrown and adult fish were subjected to serial dilutions of seawater.

Percentage concentrations of oxygen and carbon dioxide, by volume, of gas held in the gill cavities were measured after three fish, emerged for varying periods, had expelled it: an adult, 184 mm total length weighing 92.9 g; a halfgrown, 101 mm, 11.9 g; and a young, 67 mm, 3.3 g. Each fish voided bubbles through its gill slits while being gently submerged on a mechanical platform in a 12-liter aquarium half filled with a 1% solution of polyethylene oxide in seawater (Fig. 1). "Polyox" is a resin, dissolving slowly in seawater to form a non-toxic and viscous solution, which prevents the fish's ventilation and preserves any bubbles trapped in it (Todd, 1970). Expelled gas was caught in an inverted funnel filled with polyox and capped with a rubber diaphragm. Fish and funnel rested on a

platform formed of a wire frame bent to two levels about one centimeter apart and surfaced with plastic screen. The platform was smoothly raised and lowered on a rack and pinion elevator from a photographic enlarger without disturbing the fish, which rested quietly on the upper level above the liquid while the inverted funnel waited on the lower level just below the surface.

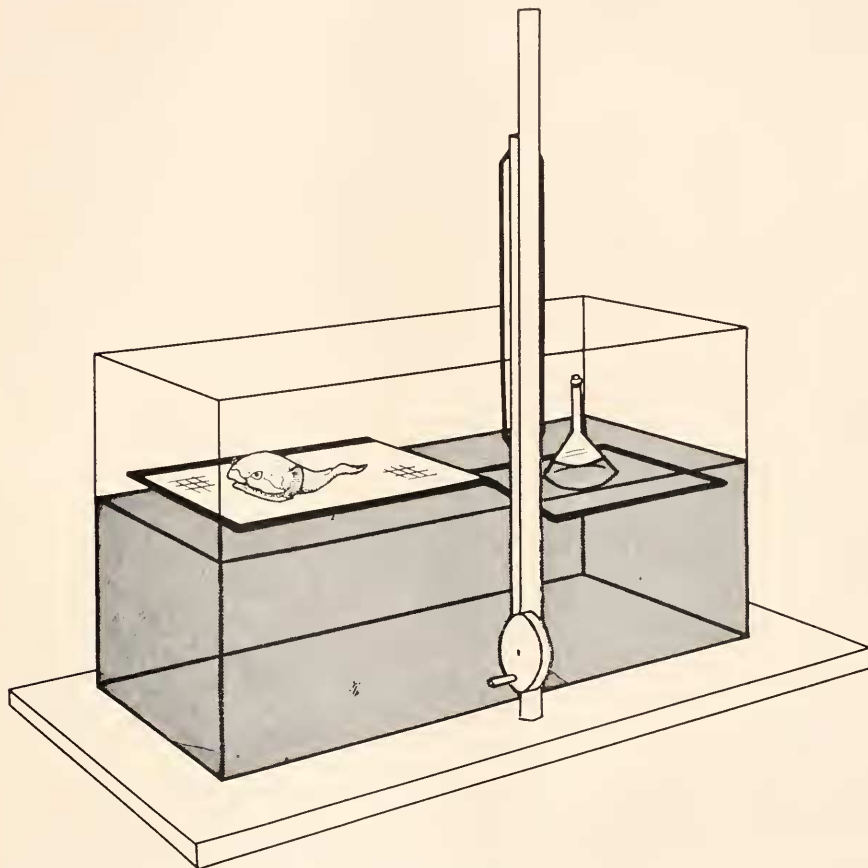


FIGURE 1. Experimental aquarium about half filled with a 1% solution of polyethylene oxide in seawater. An emerged Chilean clingfish rests above the surface on a split-level mechanical platform, which also supports an inverted funnel filled with the same "polyox" solution. Ending a specified period of emersion, the fish was slowly lowered into the liquid by cranking down the ratchet bar supporting the platform, so that the fish expelled bubbles out its gill slits into the polyox. The surfaced bubbles were preserved by the viscous polyox and captured in the funnel for later analysis of their gas content.

The fish remained quiet until slowly submerged at the end of each trial. Bubbles expelled from the gill slits were captured on the surface in the funnel, where they collected in the capped spout and were drawn into a one-milliliter syringe with dead space filled with saturated acid citrate solution. The gasping fish was quickly returned to the holding aquarium of aerated seawater, where it

soon recovered for the next trial. For the adult and halfgrown, the volume of expelled gas was estimated in the syringe; for the young, in the capillary of a Scholander microgasometer, where the gas content was analyzed (Scholander, van Dam, Claff, and Kanwisher, 1955). Because gas was usually expelled in several bubbles from both gill slits, some was probably lost and most volumetric measurements were probably minimal. Little, if any, oxygen diffused through the polyox film. The known oxygen content (15–16%) of artificially reduced gas bubbled through polyox and collected after about 30 seconds was unchanged.

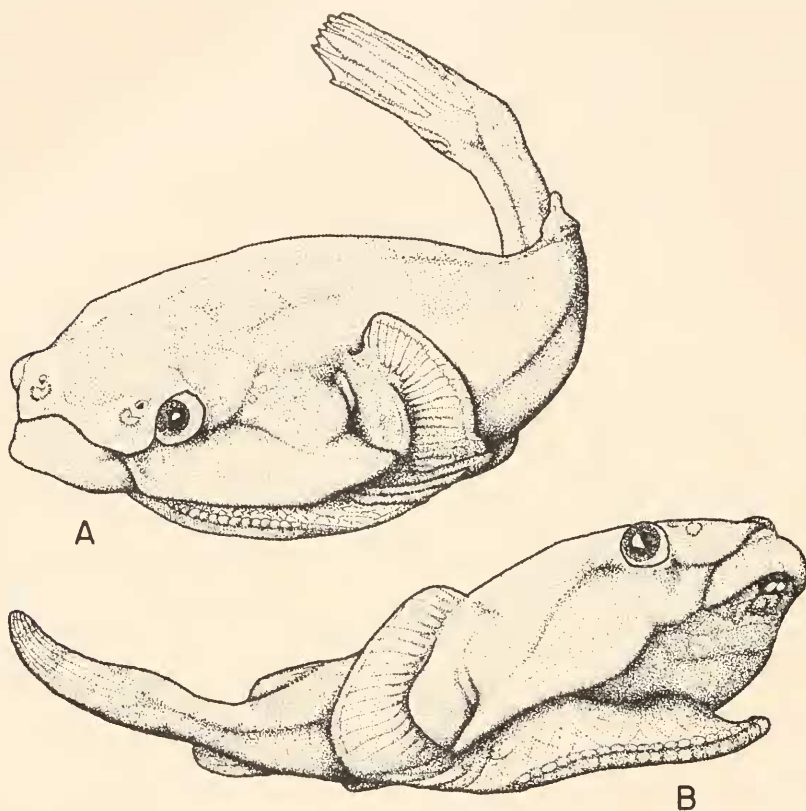


FIGURE 2. Clingfish emerged on a damp surface; A, flattened on the substrate to protect its delicate "frontal skin" on chin, leading edge of sucker, and pectoral fins; B, with head raised to expose this vasculose skin for cutaneous respiration.

Minimal rates of oxygen uptake were derived from oxygen depletion in the estimated volumes of branchial gas captured in the funnel, calculated as functions of presumed initial oxygen content (21%), content in the expelled gas, and the estimated total gas volumes (after Todd and Ebeling, 1966, where the brackets in the expression $I [(0.21 - P') - P]$ were inadvertently omitted). The initial volume (I) was taken as equal to or greater than the modal volume recorded for each fish. Volumes from the adult fish ranged from 1.4–2.5 ml, with the mode

2.2 ml. Therefore, 2.2 ml was substituted for all smaller measurements, which were probably of incomplete bubbles or bubbles emitted from one side only. All volumes exceeding 2.2 ml (2.3–2.5) were used because overestimations were unlikely. The “I” values for the halfgrown fish (0.09–0.85 ml, mode 0.20 ml) and young fish (0.01–0.04 ml, mode 0.03 ml) were estimated in the same way. Even though one value for the halfgrown fish was four times larger than the mode, it was retained as a possible measure of maximum gulping capacity.

Rates of oxygen uptake (milliliters per kilogram of wet body weight per hour) were calculated as the oxygen volumes multiplied by $1000 \div \text{weight of fish (g)}$, and by $60 \div \text{period of emersion (min)}$. Rates after the branchial gas was apparently

TABLE II
*Factors that group the variables in Table I into causal arrays**

Factor I: tranquillity		Factor III: abundance	
*Wind velocity	-0.75	*Fish, rock 1	0.99
Fish, rock 2	0.75	*Fish, total	0.97
Wave height	-0.68	Juvenile fish	-0.77
*Fish, total	0.58	Fish, seaweed fringe	0.68
Fish with heads down	0.55	Locality	0.54
*Fish, rock 1	0.46		
Factor II: water-level		Factor IV: insolation	
Dryness, upper rocks	-0.84	Air temperature	0.91
Water level	0.78	Relative humidity	-0.79
Fish, rock 3	0.78	Water temperature	0.72
Overcast	0.53	*Irradiance	0.67
Fish on lower half of rock	-0.53	Wind direction	0.48
*Irradiance	-0.48	*Wind velocity	0.48

* Variables are ordered by their relative “importance” as indicated by their loadings (numbers at right). Those marked by asterisk load “significantly” or nearly so on two factors, thereby indicating the factors’ mutual interaction. Derivation of the factors is explained in the text.

renewed during a single trial were estimated as functions of the total duration of the trial, minus the time to renewal as indicated by a sharp rise in oxygen content in previous measurements made after certain intervals: adult fish, 57 min; halfgrown fish, 35 min; young fish, 12.5 min (Fig. 3).

RESULTS

Effects of the natural environment

Communalities of the variables ordered the whole system into a hierarchy of links (Table I). The biological variables averaged higher communalities (0.71) than the physical (0.57) because they responded to several physical causes as well as interacting strongly among themselves. (The variable *fish in crevices* was excluded from the averaging because it seemed to vary randomly.) The variables of fish abundance had high communalities because emergent fish responded to short-term changes in *water level*, *dryness of upper rocks*, and *wave height*, and tended

to congregate in patterns. Physical variables like *date* and *water temperature*, on the other hand, had relatively low communalities because they probably involved long-term changes and, consequently, had little effect on the fish during the short study period. Water temperature, which varied but 3° C, may respond more to long-term fluctuations of the offshore current than to local heating during the Chilean spring. Even though they did not seem to affect fish abundances directly, the effects of insolation like *air temperature* and *irradiance* had fairly high communalities because they interacted strongly among themselves.

The factors divided the system into its component arrays of variables (Table II). The variable loadings identified the factors and revealed important environmental effects on the clingfish. The four factors were dominated by decreasing wind velocity and wave height ("tranquillity"), increasing water level with wetting of upper rocks ("water-level"), generally increasing fish abundance ("abundance"), and warming with decreasing humidity ("insolation"). For a given factor, physical variables should have the higher loadings if they evoke responses in the biological, but if biological loadings exceed physical, they may respond to other causes (Thomas, 1968). Physical variables of the tranquillity and water-level factors averaged higher loadings (0.72) than did the biological variables (0.64). Therefore, most fish came out onto wet rocks during calm periods when they often turned heads down and moved higher as the tide rose. The abundance factor, having no predominating physical loadings, simply indicated that more fish, especially adults, emerged in the more remote localities. The insolation factor, having no biological loadings, simply grouped the obvious effects of solar warming, which generated the land-sea breezes.

Several simple correlations apparently indicated indirect effects of the environment. Six variables did not load "significantly" on any factor; *i.e.*, they fell outside all four causal nexuses. *Fish in crevices*, *date*, and *barometric pressure* were apparently unimportant links within the system defined by the sampling regime. *Time of day*, *wind direction*, and *sun on rocks*, however, correlated significantly ($P < 0.05$) with several other variables in the insolation and abundance factors, which, in turn, interacted with the tranquillity factor (Table II). These simple correlations and the interactions between factors would seem to indicate that fish came out in the late afternoon when the surfaces of the observed rocks were in the sun, were it not for the multitude of fish counted during the single night station at Montemar. Perhaps more fish emerged at populous Montemar (where almost 75% of the stations were occupied) as people left the beaches in the late afternoon and, coincidentally, as sea breezes subsided and the sea calmed.

Emersion in aquaria

When halfgrown and adult clingfish emerged head first from aerated seawater, they immediately gulped air and perhaps water, stopped all opercular movement, closed their gill slits, and occasionally after several minutes turned heads down. Usually, however, they remained submerged or only partly emerged. Fish completely emerged and clinging to the smooth aquarium side gradually slipped back into the water until partly submerged. Adults placed on a horizontal platform relaxed immediately and soon appeared oblivious to laboratory activity, although emergent young moved their opercles and shifted position from time to time.

When submerged horizontally on a mechanical platform, all fish first fluttered their pectoral fins to break the suction through a groove in their ventral sucker, then moved their opercles to expel the bubbles through their gill slits.

Effects of temperature

Water warmer than 15° C disturbed submerged fish, which often clustered near the aquarium aerator and "panted" with strong and rapid opercular beats. When the seawater had warmed to an afternoon high of 17.8° C (room temperature, 20.1° C), most fish had pushed either their heads or tails above water. Those with heads submerged continued opercular beats, while those with heads out of the water had stopped all such movement.

Warm air disturbed emerged fish and seemed to elicit compensatory behavior because these fish stopped all stress reactions when subsequently cooled. An adult and halfgrown fish transferred from aerated seawater at 16.8° C to an empty aquarium in air 18.5° C closed their gill covers and were initially quiet. After a few minutes, however, the halfgrown fish began to "pant," apparently trying to pump moisture from the aquarium bottom through its gill cavities. The adult interspersed longer quiescent periods with shorter panting episodes for about an hour, then rested with gill covers closed while the halfgrown fish continued panting. At first, bubbles surrounded the opercles of both fish, as though the fish were trying to use their pharyngeal pumps. But when obviously stressed and drying fish were lightly sprinkled with seawater, they quickly relaxed and stopped panting. The halfgrown fish died within five hours with its opercles spread. The adult lived for nine hours, during which its skin became tacky. (In another trial, an adult survived 20 hours, although a halfgrown fish lived but 5 hours.)

Morphological changes accompanied the behavioral reactions to this temperature-induced respiratory stress. The pharyngeal epithelium of the emerged adult fish was reddish with vascularization and its dark red gills were engorged with blood. At one time the anterior holobranch adhered to the front of the gill chamber, while the others were clumped and pressed against the back. A thin membrane behind the chin closed the then cup-like chamber from beneath. In contrast with the pharynx, the mouth was pale to white. During active periods as the air temperature approached 18° C, the adult raised its head to expose a broad area of relatively delicate, vasculose, and unpigmented "frontal skin," extending from its chin over the broad front of its sucking disc to its pectoral fins. This area was previously hidden when the fish pressed flat to the substrate and exposed only its relatively thick, mucus-laden, and dark dorsal skin (Fig. 2A). When the fish reared its head, we saw that the frontal skin had been transformed from a white surface showing few capillaries to a reddish surface beset with conspicuous networks of engorged capillaries (Fig. 2B).

Effects of dissolved oxygen

Submerged clingfish showed no particular resistance to critically low concentrations of dissolved oxygen, although the fish usually emerged before it suffocated. In the two stagnating aquaria, adult and halfgrown fish behaved normally until the concentration fell below 2 ml/l, when all four fish had partly

emerged and had increased their opercular rates by almost 70% (Table III). While oxygen measured more than 1 ml/l, fish usually clung to the aquarium wall tails up with their heads submerged and opercles moving. Then, as oxygen continued to fall, more and more partly emerged fish clung heads up and stopped their opercular movement. While emerging, they appeared to gasp as their noses broke the water's surface. (One halfgrown fish paused with nose barely protruding the surface, while it repeatedly gulped air possibly mixed with water.) Submerged fish became restless, then breathed rapidly and laboriously for varying periods before emerging.

TABLE III
*Responses of initially submerged clingfish to decreasing dissolved oxygen**

Dissolved oxygen concentration (ml/l)	Water temperature (°C)	Position of fish						Opercular rate (beats/min)	
		Adult			Halfgrown			Adult	Halfgrown
		E/T	Pe	H/E	E/T	Pe	H/E		
5.00-7.00	12.5-13.8 (13.0)	1/16	0.1	0/1	0/10	—	—	47-120 (82)	36-160 (105)
2.00-3.51	12.3-14.6 (13.3)	4/13	0.2	0/4	0/13	—	—	46-113 (82)	55-105 (80)
1.00-1.71	14.0-15.3 (14.6)	6/15	0.1-0.6 (0.35)	0/6	2/7	0.3-0.5 (0.38)	0/2	102-205 (144)	93-203 (148)
0.45-0.89	12.2-15.8 (14.5)	11/19	0.1-0.5 (0.38)	6/11	9/14	0.1-1.0 (0.44)	3/9	121-198 (140)	105-206 (152)

* Three adult and three halfgrown fish were observed in two aquaria that were first aerated, then allowed to stagnate. For columns under "position of fish," total observations (T) included the number of sightings of fish entirely or partly emerged (E) and the number entirely submerged (T-E). The observations of emerged fish (E) included the estimated proportions of their bodies above water (Pe) and the number of fish with heads uppermost (H), as compared with the number either lying parallel with the seawater surface or with tails uppermost (E-H). Ranges of observations precede averages in parentheses.

Survival of fish in deoxygenated seawater of less than 2 ml/l varied considerably. Although one adult fish died in 4 hours, the other survived 36 hours, including 24 hours in water containing less than 1 ml/l oxygen, as it mostly rested with parts of its body above water. One halfgrown fish survived three days in water aerated but once every 24 hours.

Effects of salinity

Clingfish showed a surprising tolerance of fresh water. Four adult and halfgrown fish lived submerged without apparent distress through a series of dilutions: 24 hours in 50% seawater, then 5 hours in 25% seawater, 5 hours in 12% seawater and 24 hours in pure tapwater. Another halfgrown fish transferred directly from 100% to the 12% seawater, however, turned pale and panted for about ten minutes before returning to normal. Only this halfgrown fish and

another adult that was placed directly into tapwater died before the end of the experiment, some 58 hours after the first dilution.

Gas exchange in gulped air

Of the three clingfish emerged above the polyox solution, the adult appeared most quiescent and seemed to replenish the gas held in its gill cavities after the longest intervals. Soon after gulping air upon its removal from aerated sea-

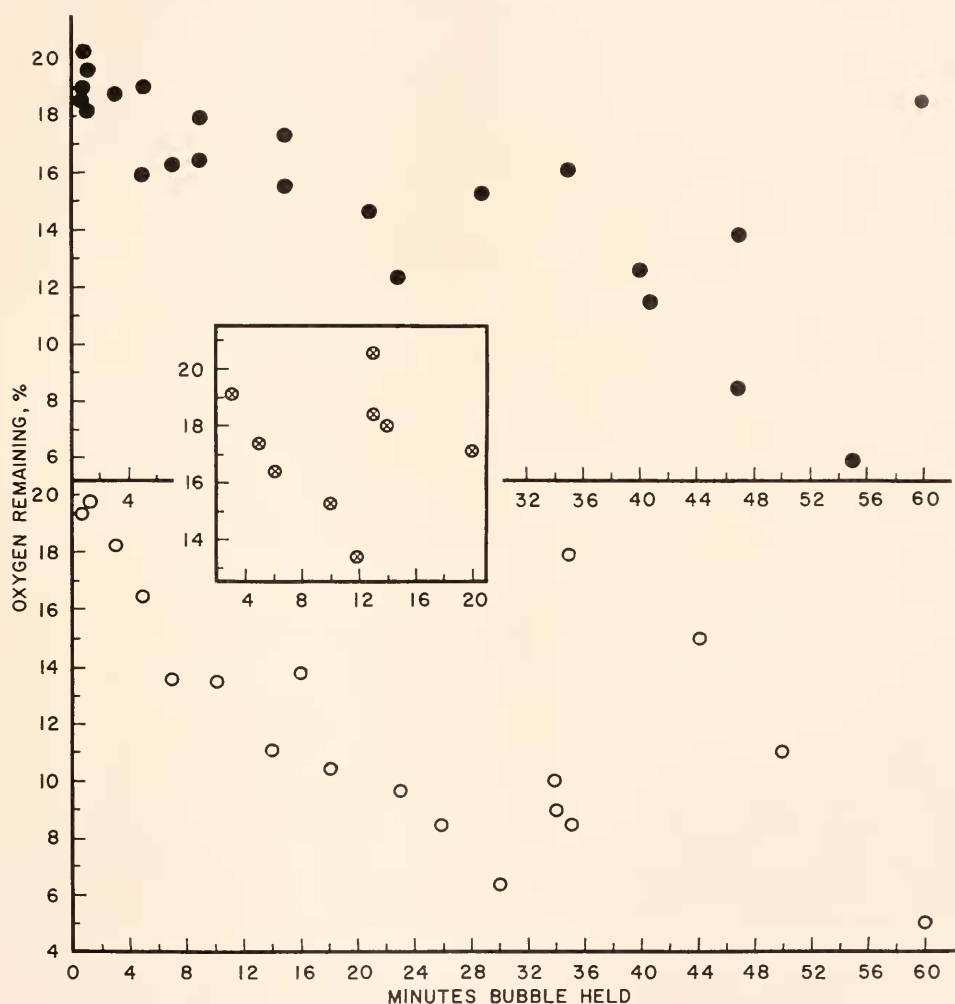


FIGURE 3. Temporal decrease in percentage oxygen in new or renewed gas held in the gill cavities of emerged clingfish; top (solid circles), expelled from a 93 g adult fish; bottom (open circles), from a 12 g halfgrown fish; inset (crossed circles) from a 3.3 g young fish. Each circle represents one measurement of the percentage oxygen in expelled gas after the fish had remained emerged on a mechanical platform for the time indicated on the horizontal axis.

water, it stopped all opercular and body movements and appeared undisturbed by activities in the laboratory. Percentage oxygen in its branchial gas expelled into the polyox solution decreased up to almost one hour, when the final observation indicated a sharp increase (Fig. 3, solid circles). This increase was unconfirmed because long experimental trials were difficult to complete. The struggling fish entering the polyox occasionally destroyed its expelled bubbles before they could be secured in the funnel, and the chance of disturbing the fish increased with time, so that reruns took up to four hours for one measurement.

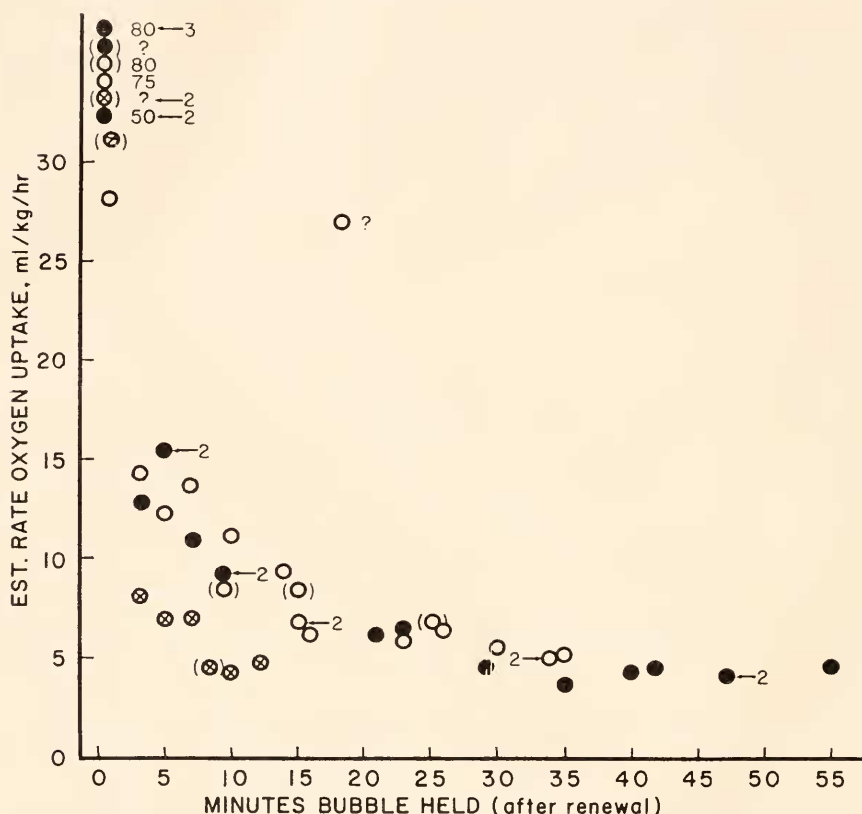


FIGURE 4. Temporal decrease in the estimated rate of oxygen uptake from gas held in the gill cavities of clingfish emerged in the experimental aquarium described in Figure 3; solid circles, adult fish; open circles, halfgrown fish, crossed circles, young fish. Parentheses enclose points depicting rates estimated from volumetric oxygen depletion after the gas had been once depleted and then renewed (see text). Circles at the upper left depict off-scale approximations of rates (numbers at right) measured after the fish had been emerged for relatively short periods of time. Problematical values are queried. Arrows indicate the average of two or three observations.

The quicker response of the halfgrown fish supported the tenuous hypothesis of gas renewal as indicated by the adult trials. Nineteen trials showed a similar sharp increase in oxygen content after slightly more than 30 minutes (Fig. 3,

open circles). Because individual trials were shorter, a complete breathing cycle was observed, showing that oxygen decreased after renewal as before: within 35 minutes, the fish had gulped, depleted, expelled, and replenished its branchial air. Like the adult, the halfgrown fish quickly relaxed on the platform, where the oxygen content of its branchial gas reached a minimum of 4-6%.

The young fish was easily disturbed, occasionally moving its opercles and frequently expelling its branchial gas. The general pattern of oxygen depletion in this gas, however, resembled the others (Fig. 3, crossed circles). Trials

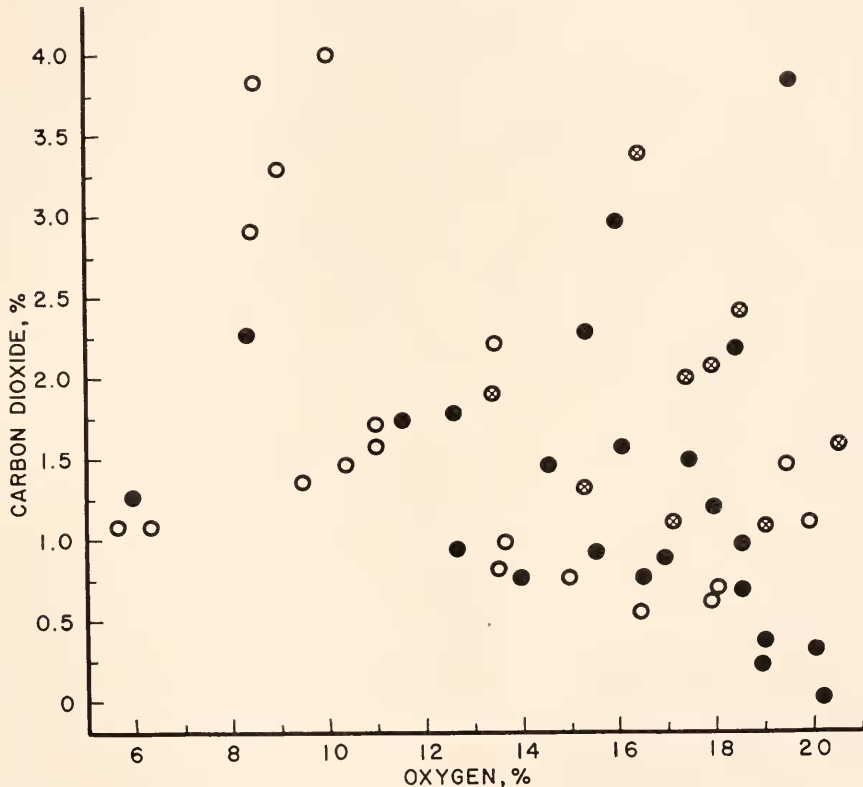


FIGURE 5. Increasing percentage carbon dioxide as a function of decreasing oxygen in gas held in the gill cavities of clingfish emerged in the experimental aquarium described in Figure 3; solid circles, adult fish; open circles, halfgrown fish, crossed circles, young fish.

lasting up to 12 minutes showed a steady decrease of oxygen to 13.4%, more than twice the minimum of the adult and halfgrown fish.

All fish held about the same relative amount of oxygen in their branchial bubbles. To compare potential oxygen supplies, ratios of modal volumes of expelled gas to body weight were divided by hours to renewal and multiplied by 1000 to rid decimals. At first glance, the adult index (25) indicated that the adult fish carried about the same supply as the halfgrown fish (29), although both indices were considerably less than that for the young fish (45). Recall,

however, that the young fish used but 36% of its oxygen, compared with 76% for the others. A corrected index of 23 ($36/76 \times 45$) showed that the young fish actually held only slightly less oxygen for its size than the others.

Rates of oxygen uptake from the branchial gas decreased precipitously during the first ten minutes of emersion, from an almost inestimable high to only 5–10 ml/kg/hr (Fig. 4). After the initial decrease, the adult rate averaged 5.3 ml/kg/hr, the halfgrown 6.8, and the young 6.2. (At about eight minutes, however, the rate of the young fish was only half that of the others.) During a 17-minute trial, the halfgrown rate was 27 ml/kg/hr (queried in Fig. 4), as calculated from an exceptionally large volume of expelled gas. Rates estimated for periods after gas renewal (points in parentheses) approximated the others.

The respiratory quotient of clingfish, as estimated from branchial gas exchange, was not unusual. Plots of carbon dioxide increase as a function of oxygen decrease, however, were scattered because the small observed percentages of carbon dioxide approached the experimental error and because several trials of less than five minutes yielded bubbles with unusually high percentages of carbon dioxide (1.5–3.8%), as though the gas were accrued near the onset of the trials (Fig. 5). The regression coefficient, which probably underestimated the respiratory quotient (cf., Carter, 1957), was 0.23, compared with 0.28 for airbreathing mudsuckers (*Gillichthys mirabilis*), as determined by Todd and Ebeling (1966). The young clingfish expelled gas containing proportionately more carbon dioxide (avg. 1.8%) than either the halfgrown (1.5%) or adult fish (1.3%).

DISCUSSION

Adaptations to optimal conditions

Factor analysis indicates that when the Chilean clingfish *Sicyases sanguineus* is in the field it avoids stress by its distribution and behavior. It positions itself so that it stays wet and relatively cool. It clings to exposed rocks continuously sprayed by the rough surf, which remains cool even on warm days. Fish even congregate on sunny surfaces so long as they are in a splash zone. The rise and fall of the water level apparently controls the vertical distribution of terrestrial fish, which cling to the upper rocks only when these rocks are splashed by high water. Beach crowds, however, may inhibit emersion of adults, even onto the preferred wet rocks with broad vertical surfaces.

Direct observations of clingfish both in the field and in the laboratory substantiate these conclusions drawn from factor analysis. In the field, fish were either restricted to the spray zone or to narrow cascades left by waves breaking over the rocks. During especially low tides, fish left the drying upper rocks. In general, they were either splashed or submerged by waves about once every five minutes and were never seen to wait longer than 15 minutes between wettings. Laboratory fish, which were not continuously splashed, often remained submerged or assumed a half-in and half-out position. Perhaps this is a compromise between total emersion without splashing, which would cause water loss and overheating, and continuous submersion, which could seem unnatural to the fish. Disturbed fish in relatively warm laboratory air appeared noticeably relieved when sprinkled with cool seawater. Like the mudskipper *Periophthalmus sobrinus* of eastern

Africa (Harms, 1935; Stebbins and Kalk, 1961), a terrestrial clingfish may normally not lose much water because it keeps its skin moist. Rao and Hora (1938) observed that a blenny, *Andamia heteroptera*, of southeastern Asia always lives above water in the intertidal spray zone, following the rising and falling tides.

It follows that the "insolation" factor does not directly affect clingfish because they avoid overheating as well as dehydration. They can markedly lighten or darken the mottled color pattern of their exposed skin, thereby controlling heat absorption. Fish held for several days in the relatively warm laboratory were generally lighter than recently captured fish, which, however, may have altered their color to blend with dark rocks in the intertidal. Stebbins and Kalk (1961) suggested that mudskippers may control their body temperature by changing skin color and avoiding midday heat. Gordon, Boëtius, Evans, McCarthy, and Oglesby (1969) presumed that for Nosy Bé mudskippers, which are protected by scales, overheating may be more hazardous than dehydration.

Survival during stress

A Chilean clingfish perhaps stranded during a violent winter storm could apparently survive substantial water loss on a drying rock, or substantial warming, stagnation, and dilution in an isolated tidal pool. Gordon, Fischer, and Tarifeño (in preparation) showed that fish survive substantial dehydration in the laboratory and Eger (in preparation) showed that other, smaller species of clingfishes tolerate surprising amounts of evaporative water loss. Even though a Chilean clingfish can survive several hours of aquatic anoxia by partial emersion, it is no more tolerant of low dissolved oxygen when it is submerged than most fishes (cf., Jones, 1964). But like the Nosy Bé mudskipper *Periophthalmus sobrinus* (Gordon, Boëtius, Boëtius, Evans, McCarthy, and Oglesby, 1965) and the estuarine mudsucker *Gillichthys mirabilis* (Todd and Ebeling, 1966) it survives in very dilute seawater. In three months of searching during the relatively dry Chilean spring, however, we found only one fish isolated in an upper tidal pool, a tiny young individual. We saw none on dry rocks. Also, we counted relatively few fish on exposed rocks during the only rainy day of the field study. Although we found no fish in bays, sheltered inlets, or river mouths, Dr. Hugo Campos of the Universidad Austral (personal communication) reportedly collected a small young individual in the Río Valdivia, several kilometers from the sea.

Adaptation to airbreathing

Airbreathing in the Chilean clingfish may have originated as a means to survive stagnation in isolated tidal pools. In general, the ability of fish to live in deoxygenated water by breathing air at the surface may constitute a preadaptation to living on land (Carter, 1957; Saxena, 1963; Johansen, 1968). Although airbreathing is rare in most groups of marine fishes, it is relatively common among species of gobies, blennies, and clingfishes that inhabit estuaries or the intertidal zone (Schöttle, 1931; Ogilaloro, 1947; Bertin, 1958; Saxena, 1963; Gordon, 1966; Eger, in preparation). And many of these species can live out of the water, escaping the intense competition and predation from the more abundant and diverse communities of subtidal predators.

The Chilean clingfish seems to have adapted behaviorally, morphologically, and physiologically to breathe air through its gills. In general, respiratory organs of amphibious teleosts include the skin, gills, mouth, pharynx, gut, and swimbladder (*e.g.*, Carter and Beadle, 1931; Carter, 1957; Krogh, 1959; Saxena, 1963; Johansen, Lenfant, Schmidt-Nielsen, and Petersen, 1968). The clingfish lacks a swimbladder and does not swallow air. Therefore, gills, skin, and buccopharyngeal epithelium are potential respiratory surfaces for emerged fish, which may consume oxygen more rapidly than submerged fish (Gordon, Fischer, and Tarifeño, in preparation). Although all three organs may contribute substantially to aerial respiration, the branchial organs seemed to be the most specialized. The tightly shut gill cavities of emerged fish may hold a mixture of air and water and serve as a kind of lung, protected from drying, sealed with liquid, and provided with a large lamellar surface covered with water for oxygen absorption. The branchial gas is not used for flotation because the fish, which lacks a swimbladder, expels the gas as it submerges and quickly dives to the bottom. The gas is not used as an oxygen store in stagnant water because it is always expelled. Vargas and Concha (1957a) observed that emerged fish with gills blocked by alginate paste survive only about one-sixth as long as control fish.

Clingfish always expelled the spent gas through their small gill slits. Under optimal conditions, furthermore, the fish's head-down position on a vertical surface would facilitate release of gas upward through their watery branchial cavities. Most other airbreathing teleosts expel such gas through their gill slits rather than out their mouth (Todd and Ebeling, 1966; Johansen, 1966; Johansen, Lenfant, Schmidt-Nielsen, and Petersen, 1968).

Relatively few fishes breathe air through their gills, because gill lamellae tend to clump in air and because oxygen is usually absorbed through some epithelium aside from the gill membrane, where carbon dioxide is most easily eliminated (Carter, 1957; Krogh, 1959; Johansen, 1966). Of those that do, a freshwater knife fish of South America, *Hypopomus brevirostris*, gulps air at the surface (Carter and Beadle, 1931), while an eel, *Symbranchus marmoratus*, often emerges from stagnant swamp waters and even hides in terrestrial burrows (Johansen, 1966). Among estuarine fishes, the mudskipper of Australia *Periophthalmodon australis* reportedly has its gills modified for aerial respiration (*cf.* Berg and Steen, 1965) and *Periophthalmus sobrinus* in eastern Africa carries mixtures of air and water in its pharyngeal and gill cavities for aerial respiration and forcefully expels the spent gas in a spray of water from its gill slits (Stebbins and Kalk, 1961). Across the Mozambique Channel, however, the conspecific Nosy Bé mudskipper apparently does not have to do this in order to breathe on land (Gordon, Boëtius, McCarthy, and Oglesby, 1969). Eger (in preparation) observed that a small amphibious clingfish of the Gulf of California, *Tomacodon humeralis*, holds bubbles of air in its moist gill cavities when it comes out of the water. Rao and Hora (1938) concluded that the terrestrial blenny *Andamia heteroptera* breathes air held in its tightly shut gill cavities.

The gills of Chilean clingfish are structurally adapted for airbreathing, more so in adult than in young fish (Vargas and Concha, 1957a). Adult fish have fewer lamellae per millimeter of gill filament and have more widely spaced lamellae on the exposed distal half of the filament than do young fish, which seem generally

less well-adapted to terrestriality. Gordon, Fischer, and Tarifeño (in preparation) noted that young fish do not survive enforced emersion as well as do adults, and the present study indicated that emergent young are much less composed than adult fish. In general, teleostean fishes that have proportionately smaller gill surfaces may survive better in air because their lamellae are more dispersed (*e.g.*, Schöttle, 1931; Gray, 1954). Indeed, all amphibious fishes that reportedly breathe air through their gills have lamellae that are widely spaced or otherwise structurally modified to prevent clumping and collapsing in air (Carter and Beadle, 1931; Schöttle, 1931; Johansen, 1966).

Clingfish out of water in laboratory aquaria waited surprisingly long times between breaths, while most of the oxygen was being depleted from the air held in their closed gill cavities. The adult fish waited for almost one hour, about twice as long as the halfgrown fish and four times as long as the young fish. Although the two larger fish had used up almost 80% of their oxygen, the young fish, occasionally moving its opercles between breaths, had used only about 40%. Johansen (1966) observed that *Symbranchus marmoratus* gulps air at the surface of deoxygenated water at varying intervals of time, averaging about 15–20 minutes while it uses up about 50% of its branchial oxygen, but occasionally extending to 30 or 40 minutes while it uses some 80% of its oxygen. The obligatory airbreathing electric eel *Electrophorus electricus* ascends at least once every two minutes (Johansen, Lenfant, Schmidt-Nielsen, and Petersen, 1968). Out of the water, the European eel *Anguilla vulgaris* inflates its gill cavities with air, which it regularly renews about once a minute at room temperature (Berg and Steen, 1966). Precht (1939) showed that cycles of airbreathing vary considerably in freshwater pulmonate snails, which may use up as much as 99% of their oxygen between breaths.

Emerged clingfish slow their heart rate and breathe at varying rates (Gordon, Fischer, and Tarifeño, in preparation). Submerged birds and mammals show a general diving syndrome including bradycardia, which prevents asphyxia and shunts oxygenated blood under pressure to the vital organs (Scholander, 1940; Andersen, 1966). Obligatory water-breathing teleosts respond in a similar way when they are emerged (Leivestad, Andersen, and Scholander, 1957; Garey, 1962). The Australian mudskipper *Periophthalmodon australis*, on the other hand, is so well adapted to life out of water that its heart beat actually slows when it is submerged, as though aquatic life were completely foreign to this fish (Garey, 1962). Like the Chilean clingfish, however, a few other amphibious fishes show some tendency toward a "diving syndrome" in air, although usually less so than typical water-dwelling fishes (Berg and Steen, 1965; Todd, 1970). In the African mudskipper *Periophthalmus sobrinus*, the response is not detectable (Gordon, Boëtius, Evans, McCarthy, and Oglesby, 1969) and in the eel *Symbranchus*, the heart rate increases after an initial period of bradycardia. Perhaps handling of the experimental clingfish intensified their bradycardia and thereby slowed their oxygen consumption (*cf.*, Kisch, 1950; Leivestad, Andersen, and Scholander, 1957), although emerged mudsuckers (*Gillichthys*) slow their heart rate markedly even with their brain removed (Todd, 1970), and mudskippers actually increase their heart rate when disturbed (Gordon, Boëtius, Evans, McCarthy, and Oglesby, 1969).

The metabolic requirements of clingfish in air may differ from those of fish in water. They breathe sporadically in air, sometimes at a faster rate than they do in water, but apparently slow down or stop breathing altogether at respiratory plateaus (Gordon, Fischer, and Tarifeño, in preparation): young fish out of water in moist respiratory chambers at room temperature consume oxygen at rates of 50–110 ml/kg/hr after 1–3 hours out of water and at 120–180 ml/kg/hr after 11–13 hours out of water; 30–80 gram halfgrown and adult fish at 2–93 (avg. 32) ml/kg/hr during 0–12 hours out of water and 4–44 (avg. 18) ml/kg/hr during 15–23 hours out of water; and aquatic young and adult fish more regularly at 32–50 (avg. 40) ml/kg/hr. Vargas and Concha (1957a) noted that the average rate of oxygen uptake at room temperature for a 120 g airbreathing adult fish, 46 ml/kg/hr, is low, compared with rates of many aquatic teleosts. It approximates the standard rate of the European eel breathing air for four hours (Berg and Steen, 1965) and of the adult mudsucker (Barlow, 1961), but approaches only half the rate of the Nosy Bé mudskipper (Gordon, Boëtius, Evans, and Oglesby, 1968). Although the European eel decreases its rate of oxygen uptake after several hours in air (Berg and Steen, 1965), *Symbranchus* actually increases its oxygen uptake in air (Johansen, 1966). Todd (1970) inferred that the mudsucker *Gillichthys* in an anoxic atmosphere can decrease its metabolism to almost zero before it finally suffocates in comparative peace.

The branchial oxygen of clingfish may sustain their lowest rates of aerial oxygen consumption, but only 12–30% of their average rate, assuming no replacement of the gas between breaths. Gordon, Fischer, and Tarifeño (in preparation), furthermore, questioned the need of fish to close their gill cavities when out of the water in their natural habitat, where they are regularly splashed by cold water. Emerged *Symbranchus marmoratus* keeps its single gill slit tightly closed for about 30 minutes, but then becomes agitated and opens its mouth to facilitate gas exchange (Johansen, 1966). When *Gillichthys mirabilis* comes out of the water, it moves its slightly opened mouth as it holds a bubble of air in its buccopharynx (Todd, 1968). Perhaps clingfish are more active and aware in the field than they are in the laboratory, and so replenish their branchial air more frequently in the intertidal splash zone. But they may have difficulty exposing their small mouth, which is inferior in position and, therefore, is usually pressed against the rock surface.

Terrestrial clingfish may also breathe through their skin. Krogh (1904) demonstrated that European eels with gills blocked can consume about 60% of their normal aquatic oxygen requirement through their skin. Berg and Steen (1965) concluded that eels consume only about one third of their total oxygen through inflated gill cavities and the rest through their skin (augmented briefly by swimbladder oxygen). Terrestrial mudskippers breathe cutaneously as well as branchially, perhaps balancing pathways without changing overall rates (Gordon, Boëtius, Evans, McCarthy, and Oglesby, 1969), and occasionally consuming some 60% of their total oxygen through their skin (Teal and Carey, 1967).

Clingfish probably breathe through their relatively delicate and vasculose "frontal skin," because the tough, thick, dark, and mucus-laden dorsal skin protecting most of their body and fins appears unsuitable for oxygen absorption (at least in adults). Fish often raise their head to expose their frontal skin, which

is shaded when they cling with head pointed downward. Other amphibious teleosts have potential respiratory epithelium located near the front of their body. Mahajan (1964) observed that the lips, barbels, and ventral adhesive pad of the Indian catfish *Glyptothorax telchitta* redden conspicuously as the emerged fish gasps air, which is forced out through its gill cavities. Vessels in the pectoral fins of airbreathing mudsuckers become noticeably engorged with blood (Todd, 1970).

Contact with air apparently stimulates clingfish to gulp. When they emerged snout first, they gulped air immediately, then closed their opercles, but when they emerged tail first, they continued their opercular movements so long as their head remained under water. Also, fish in stagnating water did not necessarily emerge and occasionally remained "panting" under water until they suffocated. Stebbins and Kalk (1961) observed that emerging mudskippers of eastern Africa quickly expand their gill cavities, then tightly close their small gill slits to secure air and water for branchial respiration on land, although Gordon, Boëtius, Evans, and Oglesby (1968) found no water in the buccopharynx and gill cavities of emerging Nosy Bé mudskippers, even though they made similar gulping movements. Johansen (1966) concluded that the most effective way to stimulate airbreathing in *Symbranchus* is simply to drain its aquarium, a much more effective stimulant than either enforced hypoxia or hypercarbia.

Unlike the Chilean clingfish, airbreathing fishes that inhabit stagnant waters of tropical swamps and coastal estuaries gulp air when they encounter varying degrees of aquatic hypoxia (e.g., Todd and Ebeling, 1966; Johansen, Lenfant, and Grigg, 1967; Johansen, 1968). Both aquatic and atmospheric hypoxia stimulate the European eel to breathe air (Berg and Steen, 1965, 1966), although only atmospheric hypoxia so stimulates the obligatory airbreathing electric eel, which does not normally come out of the water (Johansen, Lenfant, Schmidt-Nielsen, and Petersen, 1968). Willmer (1934), however, inferred that environmental concentrations of both dissolved oxygen and carbon dioxide interact to control respiration in a freshwater characid of South America, *Erythrinus erythrinus*, which breathes air through its vasculose and physostomous swimbladder. Also, Precht (1939) showed that dual effects of hypoxia and hypercarbia are additive as they stimulate and control ventilation of freshwater pulmonate snails. Various vasomotor and pressure responses may control blood flow through the respiratory organs of airbreathing fishes (cf., Steen and Kruysse, 1964; Berg and Steen, 1966; Johansen, 1968; Todd, 1970).

Relatively large proportions of carbon dioxide in the gill cavities of airbreathing clingfish did not noticeably affect their ventilation or general behavior. Concentrations as large as 4 vol. % were measured in bubbles of gas expelled from fish forcefully submerged in laboratory aquaria. Nevertheless, young fish, whose expelled gas contained proportionately more carbon dioxide than that of adult fish, were more active and easily disturbed when out of the water. Also, as emerged adults dried off, they became distressed and finally opened their gill cavities, raised their head, and panted. They seldom opened their small mouth, however, when they raised their head to expose their frontal skin.

Terrestrial fishes that are subject to drying may not be able to eliminate excess carbon dioxide from closed respiratory organs. Johansen (1966) pointed out that such fishes risk severe hypercarbia, because the normal pathway for eliminating

carbon dioxide through their gills is blocked. During enforced periods of emersion, in fact, *Symbranchus marmoratus* becomes agitated and opens its mouth after about 30 minutes. Airbreathing European eels, on the other hand, may eliminate most of their excess carbon dioxide through their moist and vasculose skin (Krogh, 1904). Gas containing more than 5 vol. % carbon dioxide passed directly over the gills of emerged eels inhibits the depth and frequency of their ventilation, although similar gas mixtures passed over their skin have no such effect (Berg and Steen, 1965). Constantly wetted by ocean spray, therefore, Chilean clingfish in the field should easily eliminate excess carbon dioxide through their skin, even while their gill cavities are tightly closed. The branchial organs of fish moistened in the laboratory showed an expectedly low respiratory quotient.

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SUMMARY

The amphibious clingfish *Sicyases sanguineus* attaches by means of its ventral sucker to vertical surfaces of large exposed rocks splashed by the cool and heavy surf of Chile and southern Perú. Although adult and halfgrown fish tolerate diluted seawater and can survive at least a few hours in warm and stagnant water, they seldom, if ever, occur in isolated tidal pools, bays, or estuaries.

Factor analysis indicates that clingfish come out of the water more abundantly during periods of calm and often turn head-down. They avoid drying rocks outside the spray zone and emerge onto higher rocks as the water level rises. Adult fish come out of the water more abundantly in remote areas relatively undisturbed by civilization. Insolation apparently does not directly alter the abundance of clingfish, which act so as to minimize evaporative water loss and overheating.

Terrestrial fish breathe air held in their gill cavities, probably through their gills. As they come out of the water, fish gulp air, then stop all opercular movements to seal their cavities, and often turn head-down. This positioning may facilitate airbreathing by easing the expulsion of spent gas upward through the watery gill cavities and by shading an area of delicate respiratory epithelium under

the chin. The volume percentage oxygen in gas expelled into a viscous solution of resin in sea water decreased regularly for about 12 minutes in a young fish, about 30 minutes in a halfgrown fish, and about one hour in an adult before these fish renewed their branchial gas. Although a concomitant increase in percentage carbon dioxide indicated that the branchial gas contributed to respiration, rates of oxygen uptake calculated from modal volumes of expelled gas were only about 12–30% of the fish's total long-term rate in air as determined by other investigators. As in other airbreathing fishes, however, cutaneous respiration may supplement branchial respiration, which fills the total need only when the metabolic rate falls.

Exposure of their head apparently stimulates clingfish to gulp, so that atmospheric air, rather than aquatic hypoxia, is their primary stimulation to air-breathing. Fish in stagnating water do not necessarily emerge and occasionally remain under water until they suffocate. Like other airbreathing fishes, clingfish appear insensitive to relatively large proportions of carbon dioxide in their branchial gas. And even with their opercles closed and their ventral mouths pressed against the substrate, clingfish in the field should easily eliminate excess carbon dioxide through their wet skin.

Vargas and Concha (1957a) emphasized the ontogenesis of terrestriality in clingfish from erratic young to well-regulated adult: modification of gill surface to minimize clumping, control of aerial oxygen uptake, and greater composure on land. The present study indicates that exposure of an anteroventral respiratory membrane, assumption of a head-down position, improvement of gulping technique, control and slowing of ventilation, and greater efficiency of exchange between branchial gas and blood also contribute critically to the maturing fish's increasing independence of aquatic life.

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